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CASE REPORT

Basophilia and megakaryoblastic differentiation in a case of acute myeloid leukemia: An unusual morphological combination

Sreejesh Sreedharanunni [a], Narender Kumar [a,*], Gaurav Prakash [b]

[a] Department of Hematology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
[b] Department of Internal Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

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Abstract
Basophilia is commonly associated with chronic myelogenous leukemia, notably in the accelerated phase or during blast crisis. It is also associated with other myeloproliferative neoplasms. However, its association with acute leukemia is very rare and is described in association with acute basophilic leukemia and few acute myeloid leukemias (AMLs) with recurrent genetic abnormalities such as t(6;9)(p23;q34). Herein, we describe the morphological features and discuss the differential diagnosis of a case of AML with the blasts showing previously unreported unusual combination of megakaryoblastic and basophilic differentiation along with peripheral blood and bone marrow basophilia.

Basophils are granulocytes that measure 10–15 μm in diameter with dark blue or purplish coarse cytoplasmic granules with a multilobed nucleus. They represent less than 2% (<0.13 × 10^9/L) of peripheral blood leukocytes in adults. Basophilia (basophils > 2%) is seen in a variety of reactive and neoplastic conditions. It is commonly associated with chronic myelogenous leukemia (CML), notably in the accelerated phase or during blast crisis. It is also associated with other myeloproliferative neoplasms such as polycythemia vera, idiopathic myelofibrosis, essential thrombocythemia, and systemic mastocytosis. Its association with acute leukemia is however very rare except in those cases of CML presenting in blast crisis [1]. We herein describe the morphological features of a unique previously

* Corresponding author at: Department of Hematology, Level 5, Research Block A, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India.
E-mail address: nkkalson@yahoo.co.in (N. Kumar).

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unreported association of acute myeloid leukemia (AML) with basophilic and megakaryoblastic differentiation and basophilia.

Case report

A 17-year-old girl presented with fever and abdominal pain of 1-week duration. The fever was of high grade and intermittent in nature, associated with chills and rigor. The abdominal pain was associated with watery diarrhea and vomiting. The patient reported a history of dyspnea on exertion, easy fatigability, palpitation, loss of weight, and appetite during the past 1 month. Physical examination revealed pallor, mild hepatomegaly, and nonsignificant cervical lymphadenopathy. There was no splenomegaly. Imaging studies of the chest and abdomen showed multiple nodules and patchy consolidation of bilateral lungs, mild bilateral pleural effusion and pericardial effusion, mild ascites, hepatomegaly, and mildly enlarged hilar and mediastinal lymph nodes. Hemogram revealed anemia (hemoglobin 76 g/L), normal leukocyte count with blasts (total leukocyte count 4.1×10^9/L; blasts 2×10^9/L), basophilia (7%; 0.3×10^9/L), and thrombocytopenia (platelet count 78×10^9/L). Peripheral blood smear showed 49% blasts, giant platelets, and circulating micromegakaryocytes. The patient underwent a bone marrow (BM) examination and biopsy. BM examination revealed cellular smears with erythroid hyperplasia (myeloid:erythroid ratio 1.3:1). Six percent of erythroid cells (Fig. 1D) showed megaloblastosis and dyserythropoiesis in the form of nuclear budding, irregularity, abnormal chromatin clumping, binucleation, and multinucleation as well as periodic Schiff-positive cytoplasmic globules. There were no ringed sideroblasts. Dysgranulopoiesis was also present in less than 10% of cells. Megakaryocytes were increased along with numerous micromegakaryocytes. Blasts constituted 26% of all nucleated cells. Nearly 20% of the blasts were positive for myeloperoxidase (MPO; Fig. 1E), but were negative for periodic acid Schiff stain on cytochemistry. Thirty percent of blasts had coarse, dark bluish purple granules, which showed metachromasia with toluidine blue stain consistent with basophilic differentiation (Fig. 1A, B, and E), whereas another 20% of blasts showed morphology suggestive of megakaryoblasts (Fig. 1A and C). These blasts were negative for MPO. Mature basophils constituted 10% of all nucleated cells in the BM. Trephine biopsy showed hypercellular marrow spaces with interstitial increase of blasts admixed with prominent number of erythroid cells. Reticulin stain showed focal fibrosis amounting to World Health Organization (WHO) Grade 1 to 2.

A multiparametric flow cytometry (FCM) was then performed. The cells were acquired on BD FACS Canto II and analyzed using BD FACS Diva software. The antibodies used were CD1a, CD2, CD3 (cytoplasmic and surface), CD4, CD5, CD7, CD8, CD10, CD11c, CD13, CD14, CD19, CD20, CD22, CD33, CD34, CD38, CD41 (surface and cytoplasmic), CD45, CD61 (surface and cytoplasmic), CD64, CD117, HLA-DR, kappa, lambda (surface), TCR$\alpha\beta$, TCR$\gamma\delta$, and TdT (BD Biosciences, San Jose, CA, USA). The gated events/cells in the blast/progenitor region ($CD45^{dim}$ positivity and low side scatter) constituted nearly 40% of all single events. These cells showed expression of CD13, CD33, CD34, CD36, CD38, CD117, and HLA-DR. Approximately 16% of the blasts also expressed CD41 and CD61, indicating megakaryocytic differentiation. The nonspecific positivity of CD41 and CD61 due to platelet aggregation on blasts was excluded, as similar numbers of megakaryoblasts were detected using cytoplasmic CD41 and CD61 as well (Fig. 2). The blasts were negative for CD11c, CD14, CD64, and B- and T-lymphoid cell markers. Multiplex reverse-transcriptase polymerase chain reaction and gel electrophoresis performed for various fusion transcripts such as *BCR-ABL1* [t(9;22)(q34;q11.2)], *RUNX-RUNX1T1* [t(8;21)(q22;q22)], *CBFβ-MYH11* [inv(16)], and

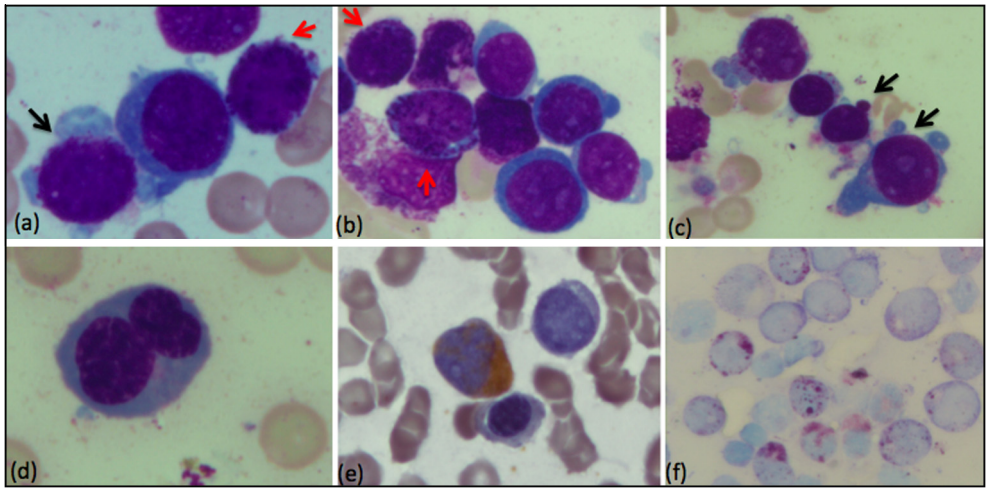

Figure 1 (A–C) Bone marrow aspirate smear showing blasts with high nuclear-cytoplasmic ratio, opened up chromatin, one to two prominent nucleoli, and basophilic granular cytoplasm. There were no Auer rods. Some of the blasts show coarse basophilic granules (red arrows), whereas some others show coarse chromatin and scanty cytoplasm with cytoplasmic blebs (black arrows) suggestive of megakaryoblasts (A–C; May–Grünwald–Giemsa stain; 100×); (D) dyserythropoiesis (May–Grünwald–Giemsa stain; 100×); (E) blasts are positive for myeloperoxidase (myeloperoxidase cytochemical stain; 100×) and (F) blasts showing metachromasia (toluidine blue stain; 100×).

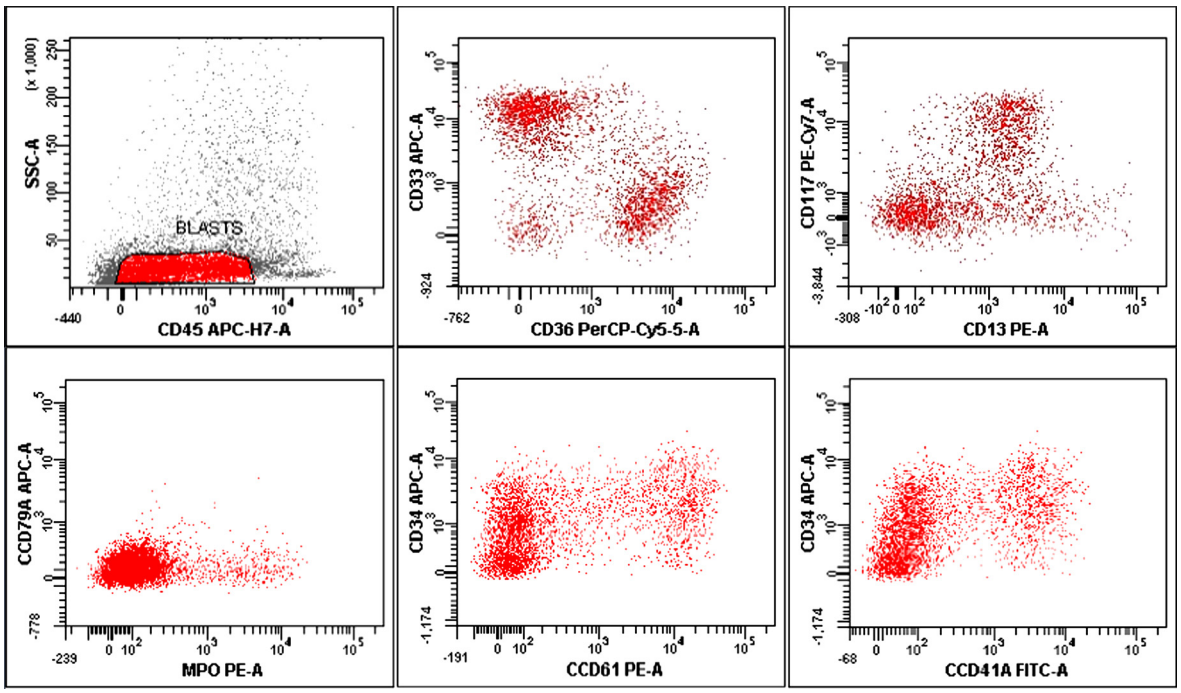

Figure 2 Multiparametric flow cytometry findings. The single events are gated in the blast/progenitor region ($CD45^{dim}$-low SSC). The same events are analyzed in subsequent plots, which showed the expression of CD13, CD33, CD34, CD36, and CD117. Approximately 16% of cells were positive for cytoplasmic CD41 and CD61; 10% cells were positive for myeloperoxidase. The cells were negative for monocytic markers (CD11c, CD14, and CD64) and B- and T-lymphoid cell markers (not shown). MPO = myeloperoxidase; SSC = side scatter.

PML-RARα [t(15;17)] did not show any recurrent molecular abnormalities. Conventional cytogenetics and karyotyping could not be performed due to technical reasons. A final diagnosis of acute myeloid/megakaryocytic leukemia with basophilia was made. Because of poor general health, the patient was not deemed fit for complete 3 + 7 induction therapy, and therefore, she was started on oral 6-thioguanine therapy along with low-dose cytosine arabinoside (10 mg/m^2 2 times a day for 10 days). However, the patient did not respond to this therapy and succumbed to the illness during the induction phase.

Discussion

The association of basophilia with AML is very rare. Literature reports have described its association with acute basophilic leukemia and few other AMLs with recurrent genetic abnormalities such as t(6;9)(p23;q34). Basophilia frequently accompanies CML in blast crisis; however, it is uncommon in *de novo* Philadelphia-positive AML [2]. Acute basophilic leukemia is a distinct entity in the WHO 2008 classification, where the blasts show primary differentiation toward basophils, and is classified under ''AML, NOS." The blasts have a characteristic morphology. They are medium sized with round or bilobed nucleus, prominent nucleoli, variable number of coarse basophilic granules positive for metachromatic stains, and cytoplasmic vacuoles. These granules have a characteristic ultrastructure and are negative for MPO, Sudan black B, chloroacetate esterase, and nonspecific esterase. AML with specific recurrent cytogenetic abnormalities needs to be excluded before making the diagnosis of acute basophilic leukemia [3]. It is important to note that unlike many other AMLs in which basophilia is noted to be associated with various translocations, mature basophils are usually sparse in acute basophilic leukemia. A few cases described in the literature as acute basophilic leukemia show basophilia [4]. Acute basophilic leukemia is distinct from AML with maturation and basophilia. Blasts will be negative for MPO in the former, whereas they will be positive in the latter [4].

AMLs with t(6;9)(p23;q34) show basophilia in nearly 60% of patients. They usually show the morphology of AML with maturation or acute myelomonocytic leukemia; however, in these cases, any of the AML subtypes can be seen other than acute megakaryoblastic leukemia or promyelocytic leukemia. These cases are often associated with multilineage dysplasia and ringed sideroblasts. They often have a high frequency of *FLT3*-ITD mutations and have a poor prognosis [3]. Patients with this translocation do not always present with AML and may present as a case of myelodysplastic syndrome or CML in blast crisis [5], t(9;22) negative CML, and myelofibrosis [6]. Other cytogenetic abnormalities reported in patients with AML and basophilia include anomalies of the short arm of chromosome 12 [7,8], t(8;21)(q22;q22) [9,10], t(X;6)(p11;q23), and novel karyotypes such as [48,XX,+del (8)(q24.2q24.3), t(21)] [6]. Basophilic differentiation is also reported in AML with myelodysplasia-related changes and complex cytogenetic abnormalities [11].

The morphology and immunophenotype of our case was consistent with AML. However, it showed a unique previously undescribed morphological combination. A fraction of blasts showed basophilic differentiation suggested by morphology and metachromatic staining; in addition,

megakaryoblastic differentiation was confirmed by FCM. There was peripheral blood and BM basophilia. The divergent differentiation of blasts as well as basophilia is described in CML in blast crisis [12]. The possibility of CML in blast crisis and *de novo* AML with t(9;22) and t(8;21) were excluded by molecular studies. There was dysgranulopoiesis and dyserythropoiesis, but these did not amount to more than 50%, which was necessary to diagnose AML with myelodysplasia-related changes. The morphological and immunological features fit best with a diagnosis of AML with basophilia and a divergent differentiation of megakaryoblast. Such heterogeneity probably depends on the cell of origin of leukemia affected by various oncogenic events. The identification of such heterogeneous population of neoplastic cells is likely to increase in future with increasing use of advanced immunophenotypic analysis. They might have a significant impact on the choice of therapeutics, minimal residual testing, and prognosis. Our report is limited by the lack of detailed cytogenetic analysis, but we aimed to highlight this previously unreported heterogenic morphological combination of basophilic and megakaryocytic differentiation of blasts in acute myeloid leukemia.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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